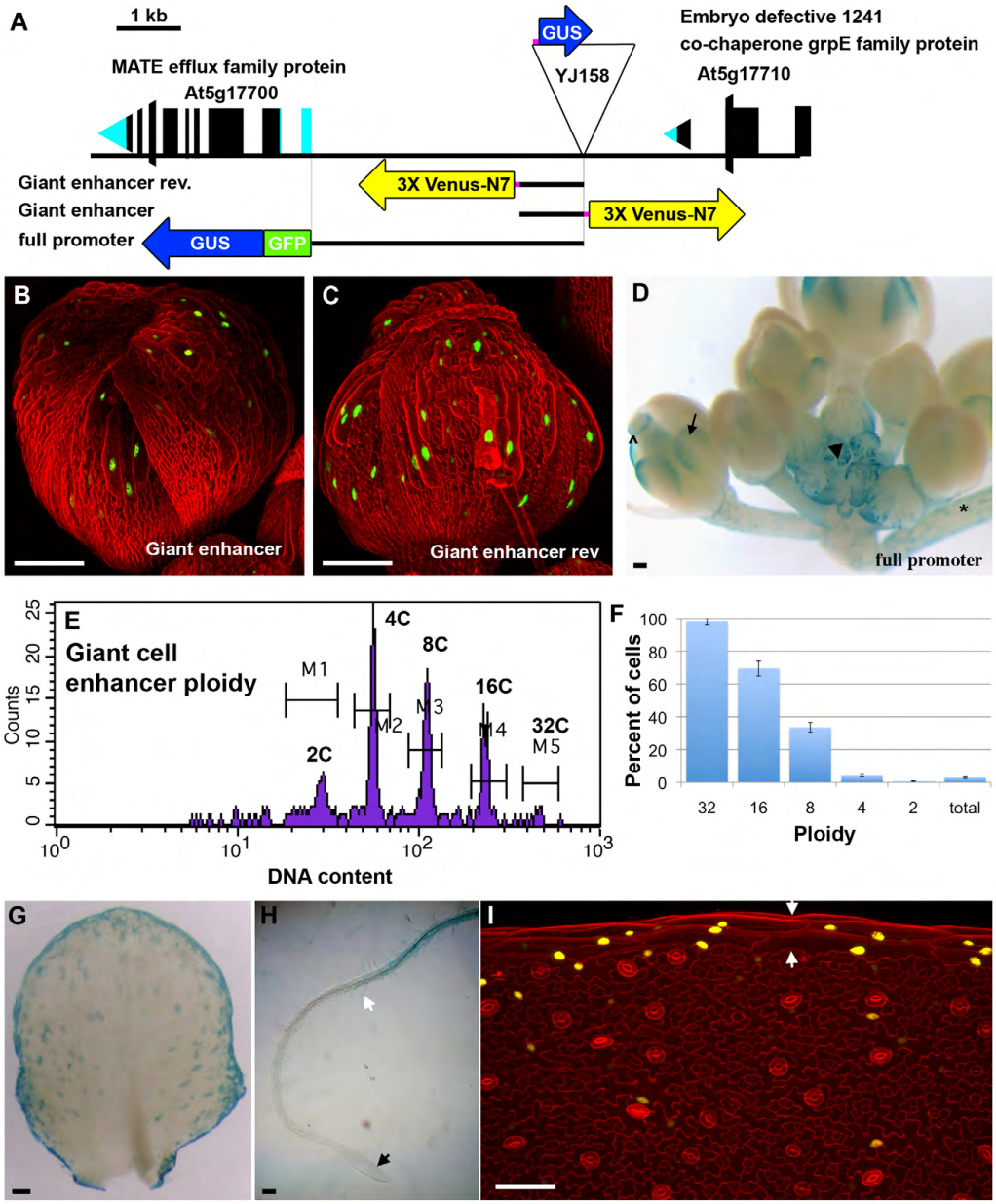

Fig. S1. A 1 kb enhancer drives reporter expression in giant cells. (**A**) The insertion site of the YJ158 giant cell enhancer trap T-DNA between the MATE efflux family protein AT5G17700 and the co-chaperone grpE protein AT5G17710 on chromosome 5. Three constructs testing enhancer elements are diagrammed below. A 1 kb region upstream of the YJ158 insertion point was tested in both orientations for ability to produce giant cell expression of the nuclear localized 3×Venus-N7 reporter (yellow arrow) with a –60 minimal 35S promoter (pink). In addition the region between the YJ158 insertion site and At5g17700 (full promoter) was tested for giant cell expression. (**B**) Expression pattern (yellow, nuclear localized) of the giant cell enhancer in the forward orientation (pAR111). The enhancer expresses in giant cells and a few smaller cells toward the tip of the sepal. (**C**) Expression pattern (yellow, nuclear localized) of the giant cell enhancer in the reverse orientation (pAR112). The expression pattern is indistinguishable from the forward orientation. (**D**) The full promoter is expressed in very young giant cells (arrowhead) as well as petal margins (arrow), the gynoecium (^), and the largest cells in the stem and pedicel (asterisk) epidermis. The sepal giant cell expression does not persist as long as that driven by the 1 kb enhancer, suggesting that the 1 kb enhancer is part of a larger regulatory unit, which modifies its expression. (**E**) DNA content of cells expressing the giant cell marker measured by flow cytometry. As expected, many giant cell marker cells are high ploidy; however, there are a few 2C and 4C cells correlating with the smaller nuclei observed in images. (**F**) The average percentage of sepal cells in each ploidy category that express the giant cell marker (*n*=4 samples of more than 22,000 cells each). Nearly all the 32C and 16C cells express the marker, whereas only a small fraction of 2C cells express the marker. Total shows the percent of all sepal cells that express the giant cell marker. Error bars represent the 95% confidence interval of the mean. (**G**) Expression of the original YJ158 giant cell enhancer trap marker in the giant cells and leaf margin cells on the abaxial side of a rosette leaf. (**H**) Expression of the original YJ158 giant cell enhancer in the mature root (white arrow). The enhancer is not expressed near the growing root meristem (black arrow). (**I**) Expression of the pAR111 giant cell enhancer in the enlarged cells of the leaf margin (cell files between white arrows) and the abaxial giant cells of the rosette leaf. Scale bars: 100 μm.

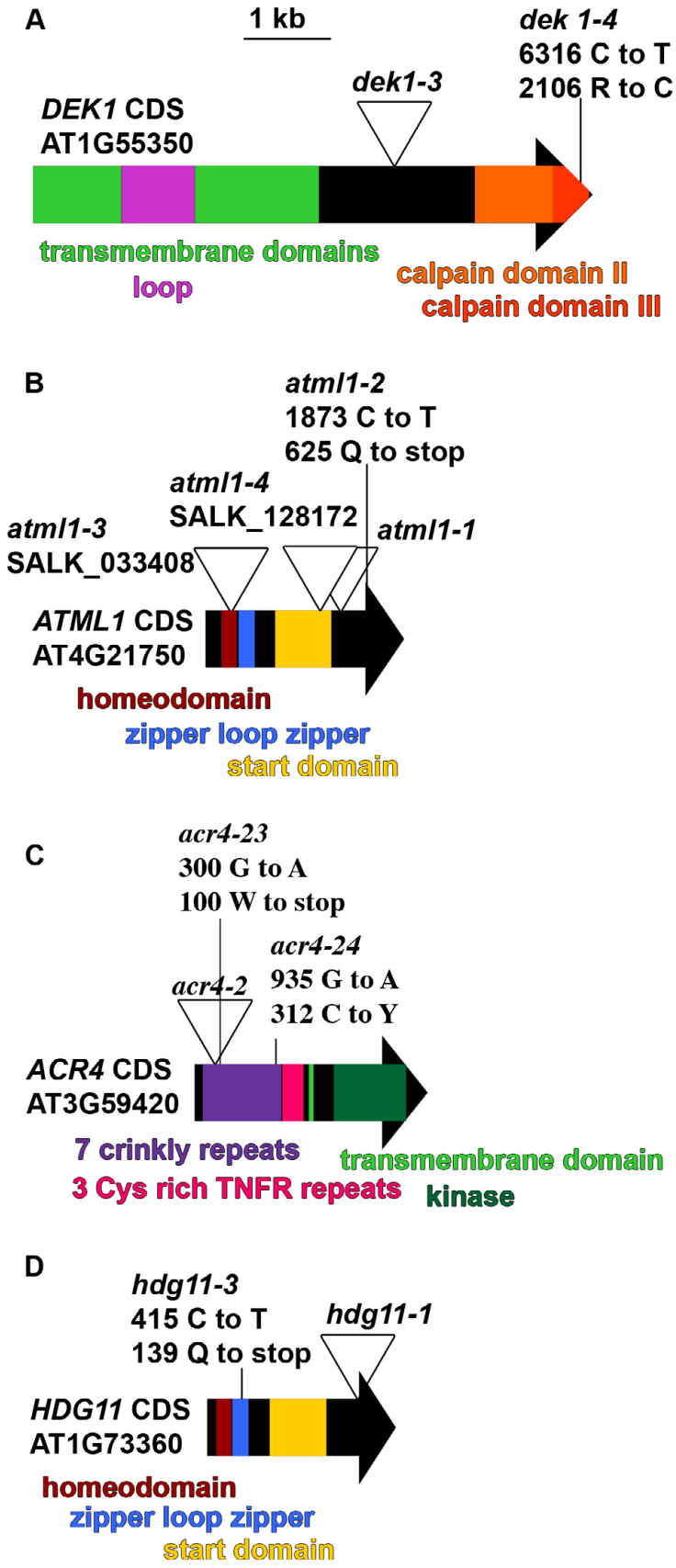

Fig. S2. Mutations in epidermal specification genes. (**A-D**) Diagrams of the cDNAs for *DEK1* (A), *ATML1* (B), *ACR4* (C) and *HDG11* (D). The regions encoding specific domains within the proteins are marked with colored boxes as labeled. The locations of the mutations in previously described and new alleles are indicated. T-DNA insertions are depicted as triangles (not to scale). Point mutations list the base changed and the affect on the amino acid sequence. Scale bar: 1 kb for all diagrams.

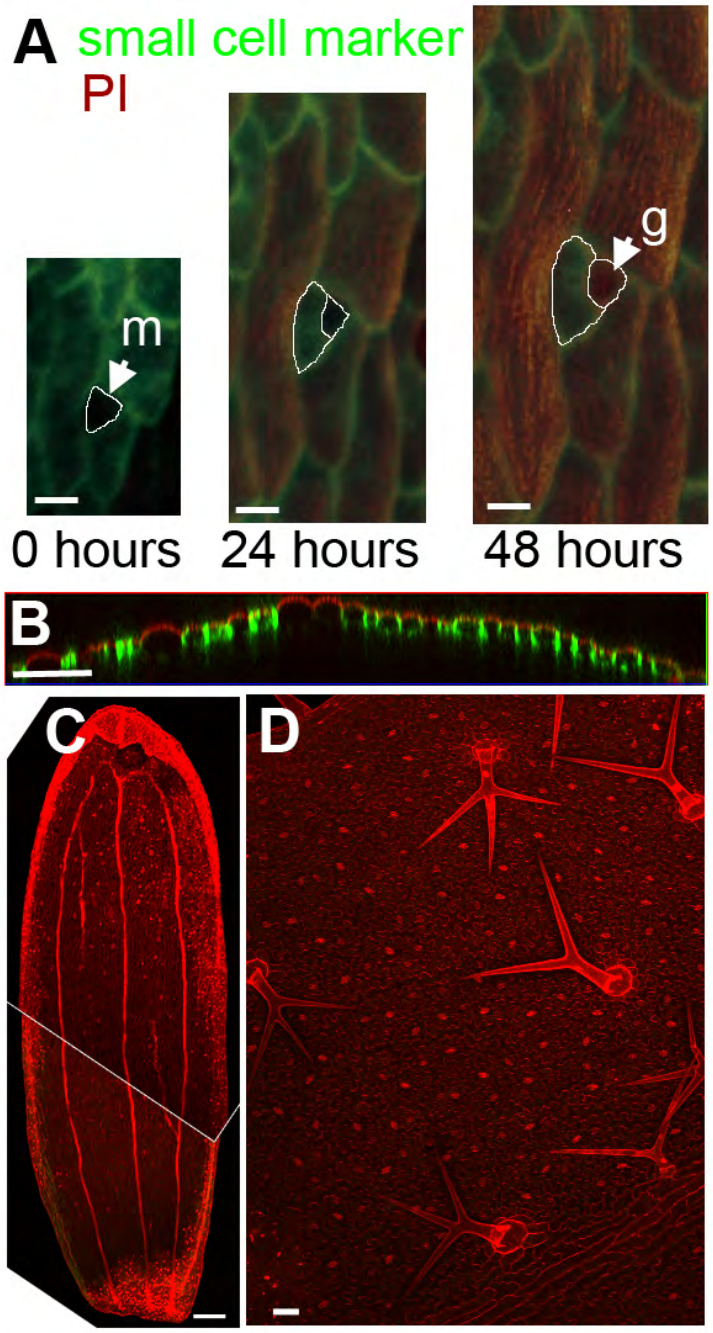

Fig. S3. Specificity of the small cell marker. (**A**) Live imaging of developing sepals expressing the small cell marker (green) and stained with PI (red) shows that the small cell marker is not expressed until after the meristemoid (white outline) cell divides. The small cell marker is not expressed in either the 2C meristemoid (arrow m) or the 2C guard cells (arrow, g). (**B**) Transverse section through a confocal image of a sepal expressing the small cell marker (green) and stained with PI (red). Note that the small cell marker is only expressed in the outer epidermis, and not the underlying mesophyll. (**C**) The small cell marker is not expressed in cells on the inner (adaxial) side of the sepal. (**D**) The small cell marker is not expressed in small cells on the bottom (abaxial) side of a cauline leaf. Scale bars: 10 μm in A; 50 μm in B-D.

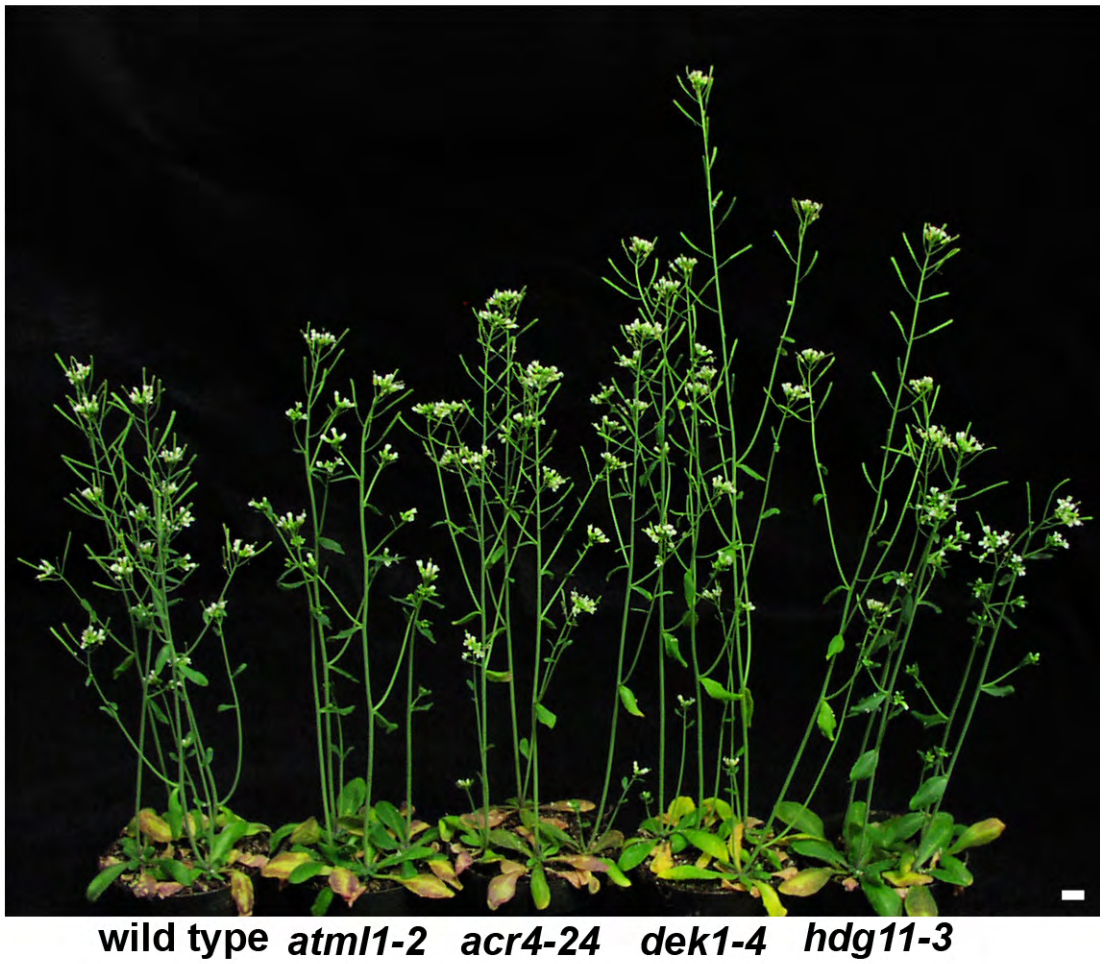

Fig. S4. Epidermal specification mutant plants appear normal. The sizes of *atml1-2*, *acr4-24*, *dek1-4* and *hdg11-3* plants are normal, although *dek1-4* plants tend to be a little taller. Scale bar: 1 cm.